CLAIMS

What is claimed is:

A method of screening a natural sample for an affinity ligand that binds to a protein target, comprising:

(1) mixing a protein target and a natural sample in solution to form a reaction mixture;

(2) incubating the reaction mixture under conditions allowing complex formation by the target and any target-binding ligand present in the sample;

- (3) passing the reaction mixture through a first size-exclusion medium that removes from the reaction mixture any small molecular weight compounds each having a molecular weight less than a first preset value;
- (4) subjecting the size-excluded reaction mixture from step (3) to conditions promoting dissociation of any ligand/target complex into free ligand and free target; and
- (5) passing the reaction mixture resulting from step (4) through a second size exclusion medium that removes from the reaction mixture any molecule larger than a second preset value.

2. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 2,000 daltons or less.

30 3. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 1,500 or less.

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WSGH Docket No. HKI-106AX WEINGARTEN, SCHURGIN, GAGNEBIN & HAYES, LLP TEL. (617) 542-2290 The method of claim 1, wherein the first size-exclusion medium comprises a gel filtration or size exclusion HPLC column.

- 5 5. The method of claim 1, wherein step (4) comprises adding to the size-excluded mixture from step (3), a solution comprising an organic solvent and an organic acid.
- 10 6. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium comprises an ultrafiltration membrane.
- 7. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.
- 8. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.
- 9. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.
- 10. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.

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- 11. The method of claim 6, wherein the ultrafiltration memorane removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.
- 5 12. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.
 - 13. The method according to claims 1, /4, or 5, further comprising:
 - (7) comparing the analytical results of step (6) with a reference standard.
 - 14. The method of claim 13, wherein the reference standard comprises the analytical results of subjecting either a sample of the protein target alone or a mixture of the protein target with a non-target-binding natural sample, to steps (2)-(6).
- 20 15. The method according to claims 1, 4, or 5, further comprising, in step (1), including a known competitive ligand that binds to the target in the reaction mixture prior to step (2).
- 25 16. The method of claim 15, wherein the concentrations of the known competitive ligand and the target are approximately equimolar.
- 17. The method of claim 15, wherein the known competitive ligand concentration is within a range of approximately twice to 10 times the target con entration.

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The method of claim 15, wherein the known competitive ligand concentration is approximately 5 times the target concentration.

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- The method of claim 15, further comprising: 19.
 - analytical comparing the of results step (6) with a reference standard.
- 20. 10 The method of claim 19, wherein the reference standard comprises the analytical results of subjecting a mixture of the protein target and the known competitive in the absence of any other target-binding ligand, ligand, to steps (2)-(6).

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The method according to claims 1, A, or 5, 21. comprising, after step (5):

subjecting the reaction mixture resulting

one

structural

least

step (5),

to functional analysis.

The method of claim 21, wherein the at least one analysis in step (6) comprises a member selected from the group consisting of mass spectrometry analysis; chromatography; liquid chromatography coupled with mass spectrometry analysis; infrared spectroscopy; nuclear magnetic resonance; an alternative binding assay; a biochemical assay; a cell-based reporter assay; ELISA-based assay.

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